


Listing being submitted concurrently herewith and to add the Sequence Listing provided herewith to the application. In addition, Applicant has amended the Specification to correct one typographical error of a forgotten subscript number. No new matter is introduced by virtue of these amendments, and the amendments are fully supported by the Specification of the subject application and the Claims as originally filed. Applicant respectfully requests that these amendments and remarks be entered and made of record in the present application.

**CONCLUSION**

No fee is believed due in connection with this submission. However, the Commissioner is authorized to charge any required fee or credit any overpayment to Pennie & Edmonds LLP Deposit Account No. 16-1150.

Respectfully submitted,

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Enclosures

**Exhibit A**

**U.S. Patent Application No. 09/760,506**

**Marked-Up Version of Amended Paragraphs**

**(Additions are double underlined, deletions are bracketed)**

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On pages 4 and 5, please replace the paragraph beginning, "Accordingly, in a first aspect" with the following paragraph:

--Accordingly, in a first aspect, the invention covers a composition comprising: (a) a saponin; and (b) an oligonucleotide comprising at least one unmethylated CpG dinucleotide. Preferably, the composition provides that the saponin is derived from *Quillaja saponaria*, and more preferably, the saponin is chemically modified or comprises a substantially pure saponin. In a preferred embodiment of the first aspect, the substantially pure saponin comprises QS-7, QS-17, QS-18, or QS-21, and more preferably, the substantially pure saponin comprises QS-21. In yet other preferred embodiments of the first aspect, the composition is further directed to one in which the oligonucleotide is chemically modified. More particularly, the oligonucleotide is modified with at least one phosphorothioate internucleotide linkage. A preferred embodiment of the first aspect encompasses the composition wherein the oligonucleotide comprises a CpG motif having the formula 5'X<sub>1</sub>CGX<sub>2</sub>3'(SEQ ID NO: 1), wherein at least one nucleotide separates consecutive CpGs, and wherein X<sub>1</sub> is adenine, guanine, or thymine, and X<sub>2</sub> is cytosine, thymine, or adenine. More preferably, the CpG motif comprises TCTCCCAGCGTGCGCCAT (SEQ ID NO: 2) or TCCATGACGTCCTGACGTT (SEQ ID NO: 3) or TCGTCGTTTTGTCGTTTTGTCGTT (SEQ ID NO: 4). The composition, according to the first aspect of the invention, preferably increases an innate immune response when administered to a mammal or a human. Still another preferred embodiment is directed to the composition wherein the composition enhances a natural killer cell response, preferably in a positive synergistic manner.--

On pages 5 and 6, please replace the paragraph beginning, "In a second aspect" with the following paragraph:

--In a second aspect, the invention is directed to a method for stimulating innate immunity comprising administering an effective amount of a composition comprising: (a) a saponin; and (b) an oligonucleotide comprising at least

one unmethylated CpG motif to an individual. Preferably, the method provides that the saponin is derived from *Quillaja saponaria*, and more preferably, the saponin is chemically modified or comprises a substantially pure saponin. In a preferred embodiment of the second aspect, the substantially pure saponin comprises QS-7, QS-17, QS-18, or QS-21, and more preferably, the substantially pure saponin comprises QS-21. In yet other preferred embodiments of the second aspect, the method is further directed to one in which the oligonucleotide is chemically modified. More particularly, the oligonucleotide is modified with at least one phosphorothioate internucleotide linkage. A preferred embodiment of the second aspect encompasses the method wherein the oligonucleotide comprises a CpG motif having the formula 5'X<sub>1</sub>CGX<sub>2</sub>3' (SEQ ID NO: 1), wherein at least one nucleotide separates consecutive CpGs, and wherein X<sub>1</sub> is adenine, guanine, or thymine, and X<sub>2</sub> is cytosine, thymine, or adenine. More preferably, the CpG motif comprises TCTCCCAGCGTGCGCCAT (SEQ ID NO: 2) or TCCATGACGTTCTGACGTT (SEQ ID NO: 3) or TCGTCGTTTTGTCGTTTTGTCGTT (SEQ ID NO: 4). The method, according to this second aspect of the invention, preferably further increases an innate immune response when administered to a mammal or a human. Still another preferred embodiment is directed to the method for further enhancing a natural killer cell response, preferably in a positive synergistic manner.--

On page 14, please replace the paragraph beginning, "One embodiment" with the following paragraph:

--One embodiment of the invention covers the oligonucleotide which contains a CpG motif having the formula 5'X<sub>1</sub>CGX<sub>2</sub>3' (SEQ ID NO: 1), wherein at least one nucleotide separates consecutive CpGs, and wherein X<sub>1</sub> is adenine, guanine, or thymine, and X<sub>2</sub> is cytosine, thymine, or adenine.--

On pages 14 and 15, please replace the paragraph beginning, "In another embodiment" with the following paragraph:

--In another embodiment, the oligonucleotide sequences useful in the methods of the invention are represented by the formula:

[5'N<sub>1</sub>XCGX<sub>2</sub>N<sub>2</sub>3'] 5'N<sub>1</sub>X<sub>1</sub>CGX<sub>2</sub>N<sub>2</sub>3' (SEQ ID NO: 5)

wherein at least one nucleotide separates consecutive CpGs; X<sub>1</sub> is adenine, guanine, or thymidine; X<sub>2</sub> is cytosine or thymine, N<sub>1</sub> is any nucleotide and N<sub>1</sub> + N<sub>2</sub> is from about 0-

26 bases. In a preferred embodiment, N<sub>1</sub> and N<sub>2</sub> do not contain a CCGG quadmer or more than one CGG trimer; and the nucleic acid sequence is from about 8-30 bases in length. However, nucleic acids of any size (even may kb long) can be used in the invention if CpGs are present, as larger nucleic acids are degraded into oligonucleotides inside cells. Preferred synthetic oligonucleotides do not include a CCGG quadmer or more than one CCG or CGG trimer at or near the 5' or 3' terminals and/or the consensus mitogenic CpG motif is not a palindrome. A "palindromic sequence" or "palindrome" means an inverted repeat (*i.e.*, a sequence such as ABCDEE'D'C'B'A', in which A and A' are bases capable of forming the usual Watson-Crick base pairs.--

On page 15, please replace the paragraph beginning, "In still another embodiment" with the following paragraph:

--In still another embodiment, the method of the invention includes the use of an oligonucleotide which contains a CpG motif represented by the formula:

5'N<sub>1</sub>X<sub>1</sub>X<sub>2</sub>CGX<sub>3</sub>X<sub>4</sub>N<sub>2</sub>3' (SEQ ID NO: 6)

wherein at least one nucleotide separates consecutive CpGs; X<sub>1</sub>X<sub>2</sub> is selected from the group consisting of GpT, GpG, GpA, ApT and ApA; X<sub>3</sub>X<sub>4</sub> is selected from the group consisting of TpT or CpT; N is any nucleotide and N<sub>1</sub>+N<sub>2</sub> is from about 0-26 bases. In a preferred embodiment, N<sub>1</sub> and N<sub>2</sub> do not contain a CCGG quadmer or more than one CCG or CGG trimer. CpG oligodeoxynucleotides are also preferably in the range of 8 to 30 bases in length, but may be of any size (even many kb long) if sufficient motifs are present, since such larger nucleic acids are degraded into oligonucleotides inside of cells. Preferred synthetic oligonucleotides of this formula do not include a CCGG quadmer or more than one CCG or CGG trimer at or near the 5' and/or 3' terminals and/or the consensus mitogenic CpG motif is not a palindrome. Other CpG oligonucleotides can be assayed for efficacy using methods described herein.--

On page 15, please replace the paragraph beginning, "In a preferred embodiment" with the following paragraph:

--In a preferred embodiment, the CpG motif comprises

TCTCCCAGCGTGCGCCAT (SEQ ID NO: 2) (also known as "CpG sequence 1758") or TCCATGACGTTCTGACGTT (SEQ ID NO: 3)(also known as "CpG

sequence 1826") or TCGTCGTTTTGTCGTTTTGTCGTT (SEQ ID NO: 4)(also known as "CpG sequence 2006").--

On pages 19 and 20, please replace the paragraph beginning, "Yet another embodiment" with the following paragraph:

--Yet another embodiment of this first aspect is directed to the oligonucleotide comprising at least one unmethylated CpG dinucleotide, wherein the oligonucleotide is modified. The particular modification may comprise at least one phosphorothioate internucleotide linkage. Further, the oligonucleotide having at least one unmethylated CpG dinucleotide may comprise a CpG motif having the formula 5'X<sub>1</sub>CGX<sub>2</sub>3', wherein at least one nucleotide separates consecutive CpGs, and wherein X<sub>1</sub> is adenine, guanine, or thymine, and X<sub>2</sub> is cytosine, thymine, or adenine. The CpG motif may preferentially be TCTCCCAGCGTGCGCCAT (SEQ ID NO: 2) or TCCATGACGTTCTGACGTT, (SEQ ID NO: 3) or TCGTCGTTTTGTCGTTTTGTCGTT (SEQ ID NO: 4).--

On pages 21 and 22, please replace the paragraph beginning, "In a second aspect" with the following paragraph:

--In a second aspect, the invention is directed to a method for increasing the innate immune response in an individual or a test system comprising administering an effective amount of a composition comprising a saponin with or without an oligonucleotide comprising at least one unmethylated CpG dinucleotide. Preferably, the saponin is a saponin from *Quillaja saponaria* Molina. More preferably, the saponin is a partially pure or a substantially pure saponin from *Quillaja saponaria* Molina. The method may also embody a composition comprising more than one substantially pure saponin and an oligonucleotide comprising at least one unmethylated CpG dinucleotide. The substantially pure saponin is preferably QS-7, QS-17, QS-18, or QS 21. Most preferably, the substantially pure saponin is QS-21. In a further preferred embodiment, the saponin may cover a chemically modified saponin or a biologically active fraction thereof obtainable from a crude *Quillaja saponaria* Molina extract. In a preferred embodiment of the method, the oligonucleotide containing at least one CpG motif is preferably a monomer or a multimer. Another preferred embodiment of the method includes the CpG motif as a part of the sequence of a vector. Yet another embodiment is directed to the method

wherein the oligonucleotide comprises at least one unmethylated CpG dinucleotide, and wherein furthermore the oligonucleotide may be chemically modified to stabilize the oligonucleotide against endogenous endonucleases. The modification may comprise at least one phosphorothioate internucleotide linkage. Further, the method may be directed, in part, to the oligonucleotide having at least one unmethylated CpG dinucleotide comprising a CpG motif having the formula 5'X<sub>1</sub>CGX<sub>2</sub>3' (SEQ ID NO: 1), wherein at least one nucleotide separates consecutive CpGs, and wherein X<sub>1</sub> is adenine, guanine, or thymine, and X<sub>2</sub> is cytosine, thymine, or adenine. In another preferred method, the unmethylated CpG motif is TCTCCCAGCGTGCGCCAT (SEQ ID NO: 2), TCCATGACGTTCTGACGTT (SEQ ID NO: 3), or TCGTCGTTTTGTCGTTTTGTCGTT (SEQ ID NO: 4).--

On page 33, please replace the paragraph beginning, "The experiments were" with the following paragraph:

--The experiments were performed using materials from the following suppliers: QS-21 and QS-7 (Aquila Biopharmaceuticals); CpG oligodeoxynucleotides included the phosphorothiate-modified sequences 1826 TCCATGACGTTCTGACGTT (SEQ ID NO: 3) and 2006 TCGTCGTTTTGTCGTTTTGTCGTT (SEQ ID NO: 4) (Life Technologies (Gibco)), murine recombinant IL-12 (Pharmingen), and YAC-1 cells (ATCC), a natural killer cell-sensitive target line.--

**Exhibit B**

**U.S. Patent Application No. 09/760,506**

**Marked-Up Version of Amended Claims**

**(Additions are double underlined, deletions are bracketed)**

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9. (amended once) The composition as claimed in claim 1, wherein the oligonucleotide comprises a CpG motif having the formula 5'X<sub>1</sub>CGX<sub>2</sub>3' (SEQ ID NO: 1), wherein at least one nucleotide separates consecutive CpGs, and wherein X<sub>1</sub> is adenine, guanine, or thymine, and X<sub>2</sub> is cytosine, thymine, or adenine.

10. (amended once) The composition as claimed in claim 9, wherein the CpG motif comprises TCCATGACGTTCCCTGACGTT (SEQ ID NO: 3) or TCGTCGTTTTGTCGTTTTGTCGTT (SEQ ID NO: 4).

24. (amended once) The method as claimed in claim 16, wherein the oligonucleotide comprises a CpG motif having the formula 5'X<sub>1</sub>CGX<sub>2</sub>3' (SEQ ID NO: 1), wherein at least one nucleotide separates consecutive CpGs, and wherein X<sub>1</sub> is adenine, guanine, or thymine, and X<sub>2</sub> is cytosine, thymine, or adenine.

25. (amended once) The method as claimed in claim 24, wherein the CpG motif comprises TCCATGACGTTCCCTGACGTT (SEQ ID NO: 3) or TCGTCGTTTTGTCGTTTTGTCGTT (SEQ ID NO: 4).

**Exhibit C**

**U.S. Patent Application No. 09/760,506**

**A List of Pending Claims upon Entry of the Present Amendment**

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1. A composition comprising:
  - (a) a saponin; and
  - (b) an oligonucleotide comprising at least one unmethylated CpG dinucleotide.
2. The composition as claimed in claim 1, wherein the saponin is derived from *Quillaja saponaria*.
3. The composition as claimed in claim 2, wherein the saponin is chemically modified.
4. The composition as claimed in claim 2, wherein the saponin comprises a substantially pure saponin.
5. The composition as claimed in claim 4, wherein the substantially pure saponin comprises QS-7, QS-17, QS-18, or QS-21.
6. The composition as claimed in claim 5, wherein the substantially pure saponin comprises QS-21.
7. The composition as claimed in claim 1, wherein the oligonucleotide is chemically modified.
8. The composition as claimed in claim 7, wherein the oligonucleotide is modified with at least one phosphorothioate internucleotide linkage.
9. (Amended once) The composition as claimed in claim 1, wherein the oligonucleotide comprises a CpG motif having the formula 5'X<sub>1</sub>CGX<sub>2</sub>3' (SEQ ID NO: 1), wherein at least one nucleotide separates consecutive CpGs, and wherein X<sub>1</sub> is adenine, guanine, or thymine, and X<sub>2</sub> is cytosine, thymine, or adenine.



10. (Amended once) The composition as claimed in claim 9, wherein the CpG motif comprises TCCATGACGTTCCCTGACGTT (SEQ ID NO: 3) or TCGTCGTTTTGTCGTTTTGTCGTT (SEQ ID NO: 4).
11. The composition as claimed in claim 1, wherein the composition increases an innate immune response when administered to a mammal.
12. The composition as claimed in claim 1, wherein the composition increases an innate immune response when administered to a human.
13. The composition as claimed in claim 1, wherein the composition increases an innate immune response when administered to a mammal other than a human.
14. The composition as claimed in claim 11, wherein the composition further enhances a natural killer cell response.
15. The composition as claimed in claim 14, wherein the composition further enhances a natural killer cell response in a positive synergistic manner.
16. A method for stimulating innate immunity comprising administering an effective amount of a composition comprising:  
(a) a saponin; and  
(b) an oligonucleotide comprising at least one unmethylated CpG motif to an individual.
17. The method as claimed in claim 16, wherein the saponin is derived from *Quillaja saponaria*.
18. The method as claimed in claim 16, wherein the saponin is chemically modified.
19. The method as claimed in claim 17, wherein the saponin comprises a substantially pure saponin.
20. The method as claimed in claim 19, wherein the substantially pure saponin comprises QS-7, QS-17, QS-18, or QS-21.

21. The method as claimed in claim 20, wherein the substantially pure saponin comprises QS-21.
22. The method as claimed in claim 16, wherein the oligonucleotide is chemically modified.
23. The method as claimed in claim 22, wherein the oligonucleotide is modified with at least one phosphorothioate internucleotide linkage.
24. (Amended once) The method as claimed in claim 16, wherein the oligonucleotide comprises a CpG motif having the formula 5'X<sub>1</sub>CGX<sub>2</sub>3'(SEQ ID NO: 1), wherein at least one nucleotide separates consecutive CpGs, and wherein X<sub>1</sub> is adenine, guanine, or thymine, and X<sub>2</sub> is cytosine, thymine, or adenine.
25. (Amended once) The method as claimed in claim 24, wherein the CpG motif comprises TCCATGACGTTTCCTGACGTT (SEQ ID NO: 3) or TCGTCGTTTTGTCGTTTTGTCGTT (SEQ ID NO: 4).
26. The method as claimed in claim 16, wherein the composition stimulates an innate immune response when administered to a mammal.
27. The method as claimed in claim 16, wherein the composition stimulates an innate immune response when administered to a human.
28. The method as claimed in claim 16, wherein the composition stimulates an innate immune response when administered to a mammal other than a human.
29. The method as claimed in claim 16, wherein the method further enhances a natural killer cell response.
30. The method as claimed in claim 16, wherein the method further enhances a natural killer cell response in a positive synergistic manner.
31. A method for stimulating innate immunity comprising administering an effective amount of a composition comprising a saponin to an individual.

32. The method as claimed in claim 31, wherein the saponin is derived from *Quillaja saponaria*.
33. The method as claimed in claim 32, wherein the saponin is modified.
34. The method as claimed in claim 32, wherein the saponin comprises a substantially pure saponin.
35. The method as claimed in claim 34, wherein the substantially pure saponin comprises QS-7, QS-17, QS-18, or QS-21.
36. The method as claimed in claim 35, wherein the substantially pure saponin comprises QS-21.
37. The method as claimed in claim 32, wherein the composition stimulates an innate immune response when administered to a mammal.
38. The method as claimed in claim 32, wherein the composition stimulates an innate immune response when administered to a human.
39. The method as claimed in claim 32, wherein the composition stimulates an innate immune response when administered to a mammal other than a human.
40. The method as claimed in claim 32, wherein the method further enhances a natural killer cell response.
41. The method as claimed in claim 40, wherein the method further enhances a natural killer cell response in a positive synergistic manner.
42. The composition as claimed in claim 12, wherein the composition further enhances a natural killer cell response.
43. The composition as claimed in claim 13, wherein the composition further enhances a natural killer cell response.